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Effects of benzylpiperazine derivatives on the acute effects of 3,4-methylenedioxymethamphetamine in rat brain

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The reduction of 5-hydroxytryptamine (5-HT) in rat brain 3 h after administration of 3,4-methylenedioxymethamphetamine (MDMA) was attenuated significantly by coadministration of benzylpiperazine derivatives (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine), which were weak inhibitors of [^{3}H]5-HT uptake into rat brain synaptosomes. However, the coadministration of desipramine and imipramine which were more potent 5-HT uptake inhibitors than these benzylpiperazines, did not attenuate the reduction of 5-HT by MDMA. These results suggest that these benzylpiperazines might inhibit the acute effects of MDMA by a novel neuropharmacological effect other than 5-HT uptake inhibition.

The administration of a single high dose of 3,4-methylenedioxymethamphetamine (MDMA) into rats causes both an immediate and a long-term reduction in brain 5-hydroxytryptamine (5-HT) concentration which is still present 1 week after administration of MDMA [2, 6–9]. The initial effects of MDMA on 5-HT concentration appear to be reversible and have been suggested to be due to enhanced release of 5-HT from nerve terminals, possibly coupled with an inhibition of transmitter synthesis [6–9]. In contrast, the long-term reduction of 5-HT in rat brain has been correlated with the neurodegeneration of serotonergic neurons [6–9]. Furthermore, the treatment with 5-HT uptake inhibitors completely blocked the immediate and long-term reductions of 5-HT in the brain after MDMA administration [1–3, 6–8], indicating that the 5-HT transporter plays an important role in the acute effects and the neurotoxicity of MDMA. It has been suggested that the protective effects of 5-HT uptake inhibitors might be attributable to their ability to inhibit 5-HT release induced by MDMA and related compounds [5]. Although the mechanisms of MDMA-induced neurotoxicity are currently unknown, the possibility of a neurotoxic basis prompted me to examine the acute neurochemical effects of MDMA.

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More recently, it has been reported that the acute and neurotoxic effects of MDMA in rat brain are attenuated significantly by coadministration of 1-piperonylpiperazine (3,4-methylenedioxybenzylpiperazine), which is a weak inhibitor of [^{3}H]5-HT uptake into synaptosomes [2–4]. At present, the pharmacological effect(s) of 1-piperonylpiperazine and the mechanism(s) underlying the antagonism by 1-piperonylpiperazine are unclear. The present study was undertaken to examine the effects of benzypiperazine derivatives on the acute effects of MDMA in rat brain. Furthermore, I compared the effects of desipramine and imipramine on the acute effects of MDMA, and also examined the effects of these drugs on [^{3}H]5-HT uptake into rat brain synaptosomes.

MDMA HCl, *p*-chlorobenzylpiperazine HCl, *p*-methoxybenzylpiperazine HCl, *p*-nitrobenzylpiperazine HCl, *o*-nitrobenzylpiperazine HCl and *m*-nitrobenzylpiperazine HCl were synthesized in our laboratory. 1-Benzylpiperazine (Wako Pure Chemical Co., Tokyo, Japan) and 1-piperonylpiperazine (Aldrich Chemical Co., Milwaukee, WI) were used as HCl salt. Other drugs were purchased commercially. Vehicle, MDMA and MDMA plus drugs were administered i.p. into male Wistar rats (200–250 g). Rats were killed by decapitation 3 h after administration, the brains were removed, and the cerebral cortex was rapidly dissected on ice and stored at −80°C until assayed. The concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral cortex was measured by high performance liquid chroma-

TABLE I

DRUG EFFECTS ON THE CONTENTS OF 5-HT AND 5-HIAA IN RAT BRAIN AFTER ACUTE ADMINISTRATION OF MDMA

Vehicle (1 ml/kg), MDMA (10 mg/kg) or MDMA (10 mg/kg) plus drugs (10 mg/kg) were administered intraperitoneally into rats. Rats were killed by decapitation 3 h after administration, and contents of 5-HT and 5-HIAA in the cerebral cortex of rats were measured by HPLC. Values are the mean ± S.D. of four rats. Values in parentheses represent percent of vehicle group.

	ng/mg Tissue	
	5-HT	5-HIAA
Vehicle (1 ml/kg)	0.20 ± 0.01	0.16 ± 0.01
MDMA (10 mg/kg)	0.03 ± 0.01 (15%)*	0.07 ± 0.01 (44%)*
MDMA (10 mg/kg) +1-benzylpiperazine (10 mg/kg)	0.04 ± 0.01 (20%)*	0.07 ± 0.01 (44%)*
MDMA (10 mg/kg) +1-piperonylpiperazine (10 mg/kg)	0.06 ± 0.02 (30%)*,+	0.08 ± 0.01 (50%)*
MDMA (10 mg/kg) +*p*-chlorobenzylpiperazine (10 mg/kg)	0.06 ± 0.01 (30%)*,+	0.08 ± 0.01 (50%)*
MDMA (10 mg/kg) +*p*-methoxybenzylpiperazine (10 mg/kg)	0.04 ± 0.01 (20%)*	0.08 ± 0.01 (50%)*
MDMA (10 mg/kg) +*p*-nitrobenzylpiperazine (10 mg/kg)	0.10 ± 0.01 (50%)*,+	0.08 ± 0.01 (50%)*
MDMA (10 mg/kg) +desipramine (10 mg/kg)	0.04 ± 0.01 (20%)*	0.08 ± 0.02 (50%)*
MDMA (10 mg/kg) +imipramine (10 mg/kg)	0.05 ± 0.01 (25%)*	0.07 ± 0.01 (44%)*

*$P < 0.01$ when compared with vehicle group. +$P < 0.01$ when compared with MDMA alone group.

tography (HPLC) with electrochemical detection as described previously [1, 2]. The IC_{50} values of drugs on [^{3}H]5-HT uptake into the synaptosomes prepared from the whole rat brain were measured as described previously [2]. The statistical evaluation of the multiple data was performed by a one-way analysis of variance, followed by the Fisher's PLSD test.

Table I shows the effects of drugs on the reduction of 5-HT and 5-HIAA in the cerebral cortex 3 h after a single administration of MDMA. The contents of 5-HT and 5-HIAA in the cerebral cortex after administration of MDMA (10 mg/kg) were decreased to 15% and 44% of control, respectively. The coadministration of 1-piperonylpiperazine (10 mg/kg), *p*-chlorobenzylpiperazine (10 mg/kg) and *p*-nitrobenzylpiperazine (10 mg/kg) significantly attenuated the reduction of 5-HT produced by MDMA. However, the coadministration of 1-benzylpiperazine (10 mg/kg), *p*-methoxybenzylpiperazine (10 mg/kg) and desipramine (10 mg/kg) did not significantly alter the reduction of 5-HT produced by MDMA. Although the coadministration of imipramine (10 mg/ kg) slightly attenuated the reduction of 5-HT produced by MDMA, the change was not statistically significant. Furthermore, the reduction of 5-HIAA in the cerebral cortex by MDMA was not altered by coadministration of all drugs examined.

Table II shows the effects of *o*-, *m*-, *p*-nitrobenzylpiperazines on the immediate reduction of 5-HT and 5-HIAA in the rat brain by MDMA. The coadministration of *p*-nitrobenzylpiperazine (10 mg/kg and 20 mg/kg) significantly attenuated the reduction of 5-HT produced by administration of MDMA (10 mg/kg). The administration of a high dose (20 mg/kg) of *p*-nitrobenzylpiperazine completely inhibited the reduction of 5-HT by MDMA. At the dose used in this study, neither *o*- or *m*-nitrobenzylpiperazine blocked MDMA-induced 5-HT depletion in the cerebral cortex. The concentration of 5-HIAA in the cerebral cortex 3 h after administration of MDMA was not altered significantly by coadministration of these three compounds. Furthermore, a single administration of these compounds (10 mg/kg) did not alter the content of 5-HT in the cerebral cortex, but the content of 5-HIAA was decreased significantly by *p*-nitrobenzylpiperazine (10 mg/kg).

Table III shows the IC_{50} values of drugs on [^{3}H]5-HT uptake into the synaptosomes prepared from the whole rat brain. The benzylpiperazine derivatives examined were weak 5-HT uptake inhibitors, being 10- or 10^2-fold less potent at inhibiting [^{3}H]5-HT uptake as compared with desipramine or imipramine, respectively. Thus, it is likely that these benzylpiperazine derivatives are very weak inhibitors of [^{3}H]5-HT uptake into synaptosomes.

The major finding of the present study is that the acute effects of MDMA were attenuated significantly by coad-

TABLE II

EFFECTS OF *o*-, *m*-, *p*-NITROBENZYLPIPERAZINES ON THE REDUCTION OF 5-HT AND 5-HIAA IN RAT BRAIN AFTER ACUTE ADMINISTRATION OF MDMA

Vehicle (1 ml/kg), MDMA (10 mg/kg), MDMA (10 mg/kg) plus drugs (10 mg/kg or 20 mg/kg) and drugs (10 mg/kg) were administered intraperitoneally into rats. Rats were killed by decapitation 3 h after administration, and the contents of 5-HT and 5-HIAA in the cerebral cortex of rats were measured using HPLC. Values are the mean ± S.D. of four rats. Values in parentheses represent percent of vehicle group.

	ng/mg Tissue	
	5-HT	5-HIAA
Vehicle (1 ml/kg)	0.15 ± 0.04	0.20 ± 0.04
MDMA (10 mg/kg)	0.03 ± 0.01 (20%)**	0.10 ± 0.03 (50%)*
MDMA (10 mg/kg) +*o*-nitrobenzylpiperazine (10 mg/kg)	0.05 ± 0.02 (33%)**	0.08 ± 0.01 (40%)*
MDMA (10 mg/kg) +*m*-nitrobenzylpiperazine (10 mg/kg)	0.05 ± 0.01 (33%)**	0.08 ± 0.01 (40%)*
MDMA (10 mg/kg) +*p*-nitrobenzylpiperazine (10 mg/kg)	0.10 ± 0.01 (67%)*,+	0.10 ± 0.01 (50%)*
MDMA (10 mg/kg) +*p*-nitrobenzylpiperazine (20 mg/kg)	0.16 ± 0.02 (107%)++	0.13 ± 0.01 (65%)*
o-nitrobenzylpiperazine (10 mg/kg)	0.17 ± 0.05 (113%)	0.24 ± 0.01 (120%)
m-nitrobenzylpiperazine (10 mg/kg)	0.16 ± 0.05 (107%)	0.23 ± 0.01 (115%)
p-nitrobenzylpiperazine (10 mg/kg)	0.18 ± 0.05 (120%)	0.13 ± 0.02 (65%)*

*$P < 0.05$, **$P < 0.01$ when compared with vehicle group. +$P < 0.05$, ++$P < 0.01$ when compared with MDMA alone group.

ministration of benzylpiperazine derivatives (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine). The coadministration of desipramine and imipramine did not attenuate significantly the acute effects of MDMA, although desipramine and imipramine are about 10- and 10^2-fold more potent 5-HT uptake inhibitors as compared with these benzylpiperazines, respectively. Imipramine for blocking the acute effects of MDMA was less active than these three benzylpiperazines (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine), although imipramine was about 10^2-fold more potent in inhibiting [^{3}H]5-HT uptake as compared with these three benzylpiperazines, suggesting that these benzylpiperazines might inhibit the acute effects of MDMA by effect(s) other than 5-HT uptake inhibition. Thus, these benzylpiperazines may represent a new class of compounds which inhibit the acute effects of MDMA. Among different substitutions made on the phenyl ring, the *p*-nitro group which has an electron-withdrawing effect was most potent. Interestingly, it has been found that *p*-trifluoromethylbenzylpiperazine, a drug having the trifuoromethyl group which has an electron-withdrawing effect, attenuated significantly the reduction of 5-HT in rat brain by MDMA (Hashimoto, unpublished). Moreover, the coadministration of raclopride (dopamine D_2 antagonist), SCH 23390 (dopamine D_1 antagonist), nomifensine (dopamine uptake inhibitor), ketanserin (5-HT_2/5-HT_{1c} antagonist), ICS 205–930 (5-HT_3 antagonist), piperoxan (α_2 adrenergic antagonist), propranolol (β adrenergic antagonist), MK 801 and ketamine (*N*-methyl-D-aspartate antagonists), pyrilamine (histamine H_1 antagonist), cimetidine (histamine H_2 antagonist) and nicardipine (calcium channel antagonist)(5 mg/kg) did not alter the reduction of 5-HT in the brain produced by MDMA (10 mg/kg) (Hashimoto, unpublished data). These data suggest that these benzylpiperazines might not possess these pharmacological effects examined in these experiments. Thus, it appears that these benzylpiperazine derivatives may pos-

TABLE III

DRUG EFFECTS ON [^{3}H]5-HT UPTAKE INTO RAT BRAIN SYNAPTOSOMES

The IC_{50} values of drugs on [^{3}H]5-HT uptake into rat brain synaptosomes were measured as described previously [2]. The values are the mean of three determinations done in duplicate, the S.D. of which are less than 10%.

	IC_{50} (μM)
1-Benzylpiperazine	53.7
1-Piperonylpiperazine	55.6
p-Chlorobenzylpiperazine	25.9
p-Methoxybenzylpiperazine	60.3
p-Nitrobenzylpiperazine	15.8
Desipramine	2.96
Imipramine	0.421

sess novel neuropharmacological effect(s) in the central nervous system.

It has been reported that 5-HT uptake inhibitors such as fluoxetine can be administered up to 6 h following MDMA and still protect against the neurotoxicity of MDMA in the 5-HT neurons [6]. These data suggest that the release of 5-HT by itself is not neurotoxic, since the immediate effect of MDMA is the release of 5-HT from nerve terminals. However, we reported recently that these benzylpiperazine derivatives (*p*-nitrobenzylpiperazine, *p*-chlorobenzylpiperazine and 1-piperonylpiperazine) significantly attenuated the neurotoxicity of MDMA in rat brain, and that the potencies of these benzylpiperazines in blocking the acute effects of MDMA were highly correlated with the potencies in blocking the MDMA-induced neurotoxicity [4], suggesting that the inhibition of the acute effect by these benzylpiperazines might lead to the inhibition of MDMA-induced neurotoxicity. Moreover, the coadministration of *p*-nitrobenzylpiperazine attenuated significantly the immediate reduction of 5-HT in the brain after administration of other amphetamine derivatives (*p*-chloroamphetamine and fenfluramine) (Hashimoto, unpublished). At present, the mechanism(s) underlying the inhibition of acute and neurotoxic effects of MDMA by these benzylpiperazines are unknown. It is well known that the acute neurochemical effects of MDMA are its carrier-mediated release of 5-HT [5, 7]. Therefore, it is likely that the 5-HT transporter may play a significant role in the inhibition of acute effects of MDMA by these benzylpiperazines, although these benzylpiperazines are weak inhibitors of [^{3}H]5-HT uptake into synaptosomes. Interestingly, I have found that specific in vivo binding of [^{3}H]6-nitroquipazine to the 5-HT transporter in the brain is decreased significantly by pretreatment with *p*-nitrobenzylpiperazine, whereas not by pretreatment with 1-benzylpiperazine (Hashimoto, unpublished data). Unlike 5-HT uptake inhibitors, these benzylpiperazines may regulate the 5-HT transporter in vivo by acting at recognition sites on 5-HT nerve terminals, although the exact site(s) at which these benzylpiperazines act remain to be elucidated. Further studies on this hypothesis are necessary. In conclusion, *p*-nitrobenzylpiperazine would be a suitable drug for studying the acute effects of MDMA and related compounds.

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